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Nutrition Rehabilitation of the HIV-Infected and Negative Undernourished Children Utilizing Spiruline

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Abstract: The objective of the study was to assess the impact of an alimentary integrator composed by Spiruline (*Spirulina platensis*), produced at the Centre Medical St Camille (CMSC) of Ouagadougou, Burkina Faso, on the nutritional status of undernourished HIV-infected and HIV negative children. We compared two groups of children: 84 children HIV-infected and 86 HIV-negative. The duration of this study was eight weeks. Anthropometrics and haematological parameters allowed us to appreciate both the nutritional and biological effects of Spiruline supplement to traditional meals. The rehabilitation with Spiruline shows on average a weight gain of 15 and 25 g/day in HIV-infected and in HIV negative children, respectively. The level of anaemia decreased during the study in all the children, but the recuperation was less efficient among the HIV-infected children, in fact, 81.8% of HIV negative undernourished children recuperated against 63.6% of HIV-infected children [Z: 1.70 (95% CI: -0.366, -0.002, p=0.088)]. Present results allow to confirm that Spiruline is a good food supplement for undernourished children. In particular the rehabilitation by Spiruline seems to correct the anaemia and the weight loss also in HIV-infected, but more quickly in HIV negative undernourished children.

Key words: Malnutrition, Spiruline, HIV, Burkina Faso

INTRODUCTION

Malnutrition constitutes a public health problem in all the world particularly in the developing countries[1]. In Africa, more than 30% of the infant mortality rate below five years of age results directly or indirectly from malnutrition[2]. Since 1999, Burkina Faso is confronted by protein-energetic malnutrition with 13% of infant population affected by emaciation, 29% by growth delay and 30% by insufficient weight[3]. The consequences of the protein-energetic malnutrition in Burkina Faso are several and manifest severe forms of marasma, kwashiorkor and kwashiorkor-marasma[3]. To day it is recognized that this form of malnutrition is coupled with deficiencies in vitamins and in minerals[4,5]. It creates inexorably an uncorrectable spiral between malnutrition and infectious pathologies, which often is associated with chronic diarrhoea[6]. The more frequent infection found in severe undernourished children is the HIV infection, which occupies an important place in this country. In fact, HIV prevalence of 40.5% was recognized in undernourished children[3] and compounds the prognosis of these children.

In Centre of Education and of the Nutritional Rehabilitation (CREN) of Ouagadougou, Burkina Faso, a supplement with Spiruline was used since January 2000 to improve the nutritional status of undernourished HIV-infected and HIV negative children. The choice of this alimentary integrators, a cyanobacterium which growth easily at the temperature of Burkina Faso, was guided by the biochemical composition in aminoacids, iron, carotenoids of the Spiruline powder. Spiruline has been recently introduced in the treatment of HIV infected undernourished children because of several advantages of Spiruline in the rehabilitation HIV adult patients have been reported[7,8]. Spiruline used in this study was also analysed for its glucid and lipid compositions since they are influenced by the environmental condition of growth.

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MATERIALS AND METHODS

Study protocol: This research was conducted at the CMSC of Ouagadougou during 2002-2003. This Center was created in 1974 by the religious of St Camille order and comprises a maternity, a health center, an analysis laboratory for biological and biochemical examination, a Center for Neonatal Pathology, a greenhouse for the culture of the Spiruline and a Center for Education and for Nutritional Rehabilitation (CREN). The CREN follows in average 700 children for year.

Infants and children aged <5 years HIV-infected and HIV negative were enrolled using the CONSORT criteria[9]. Dehydrated children in shock needing rapid transfer to hospital for intensive therapy, were excluded from this study. Each child was admitted to the protocol study given a progressive number and at the end each was selected with a casual number generator program. This procedure was repeated separately for each group. All parents provided written consent for participation of their children in the study protocol.

Study patients: At the beginning of this study, undernourished children were anhoressic and many of them had diarrhoea, which was treated with nose-gastric rehydratation according the CMSC protocol[6]. The nose-gastric rehydration was interrupted before inclusion in this study. At the end 170 children (84 HIV infected and 86 HIV negative chidren) were randomly enrolled in four rehabilitation protocols: B) 44 HIV infected children were given the supplement Spiruline together with the traditional meals (millet, vegetable, fruit); A) 46 HIV negative children were treated by the supplementation of Spiruline to traditional meals (millet, vegetable, fruit); D) 40 HIV infected children of the same age range were given only with traditional meals; C) 40 HIV negative children were alimented only with traditional meals. The group C and D represent the control group for Spiruline supplement.

No antiretroviral treatment was used in HIV-infected children because it was not available. The vitamin and mineral deficiencies were corrected only at the end of study.

Participation criteria: All studied children were undernourished according to the z-score criteria, recommended by the WHO and the Funds of the United Nations for l'Enfance (UNICEF) and their age was 14.37 (range 12-60 months). The ages were confirmed by the birth notebooks. The Ethical Committee of CMSC gave permission for the study. The exclusion criteria were refusal to participate to the study, while the discontinuation of participation criteria were abandonment, death and the interruption of treatment at the Center during the study.

Anthropometrics parameters: The weight of the children was taken once a week since the day of their admission to the CREN with a 10 g sensitivity balance. The height of children under 2 years is measured by resting the child on his back; those children over 2 years is measured in the upright position. The nutritional status, evaluated by brachial perimeters was compared to the classification of Jelliffe[10], considering that it varies little for the children of less than four years.

HAZ (Height for age z-score), WHZ (Weight for height z-score) and WAZ (Weight for age z-score) parameters were calculated according to the references of the National Center for Health Statistics (NCHS)[11].

Evaluation of results: The evaluation of nutritional status of the children has been made according to the nutritional indices. The index weight for age expressed in z-score (WAZ) or weight insufficiency translates a global malnutrition affecting at once the linear growth and the weight increment. The index height for age expressed in z-score (HAZ) or growth delay is an index that translated a chronic malnutrition provoked by an extended reduction of the food consumption and by repeated pathologic episodes. The emaciation or weight loss expressed by the index weight for height (WHZ) indicates a slighter status or weight deficit due to a decrease, or slowdown of regular growth. These tests were performed to detect significant changes within the treatment groups in order to assess whether Spiruline is a useful supplement of feeding in rehabilitation.

Biological analyses: The level of haemoglobin, number of leukocytes, lymphocytes and neutrophiles were measured before and after eight weeks from the starting of the study only among HIV-infected and HIV negative children treated with Spiruline, because our priority objective was to demonstrate the efficacy of this treatment in nutritional rehabilitation of HIV-infected and HIV negative children. The blood numeration was made using a Coulter Counter T540. The haemoglobin level and the hematocrite were measured in order to reveal an anaemia status. The number of lymphocytes, neutrophiles and leukocytes gives an idea of the severity of immune involvement. An anti HIV antibodies test was done with Biorad Genie II Rapid Test (USA) on children, whose parents freely accepted our research protocol. All of our

Fig. 1: Particular of bacins for the Spiruline cultivation

positive samples were tested with the technique of EIA (Enzyme Immuno Assay) using Abbott IMX System (USA) in order to confirm the samples HIV-infected .

Plant material: Spiruline was cultivated in Burkina Faso, in artificial ponds and dried at room temperature (Fig. 1). The material was stored in the dark at 4°C to prevent photo degradation.

Preparation and administration of the Spiruline: The mothers of the undernourished children which received Spiruline were given weekly rations of 70 g of Spiruline in a sachet. Every day, they had to mix 10 g of Spiruline with a graduated container to the traditional meal of their children composed of millet and flour. This mixture was made at least two times a day. This mixture was given to children in a quantity covering their caloric requirements and outside the suckling moments in children whose mothers continued breast feeding. The compliance of mothers on how prepare mixes and feed them to their children was elevated and they continue to administrate the mixture also at home. Each week they accompany children in the CREN for a control of weight and other anthropometric parameters.

Fatty acids quantification and identification: The Spiruline was ground and extracted three times with hexane. The mixture of fatty acid methyl esters has been extracted with hexane and analyzed by Hewlett Packard gas-chromatograph, Model 5890, equipped with a Flame Ionization Detector (FID) and coupled to an electronic integrator. The components were identified by using standard fatty acid methyl esters and quantified by using methyl nonadecanoate (19:0) as an internal standard.

Statistic analysis: A power analysis was performed prior to the initiation of the study and the number of studied children was homogeneously distributed and reached the minimal number to discuss a statistical difference. The data were treated with Excel (Office, Microsoft) software, Epi-Info software V. 6 for the anthropometrics data and SPSS-10 for biological data, according to the opportunities of calculations and of analysis. The difference between mean values before and after eight weeks of treatment were calculated by Student t-test (for paired and unpaired data). $p<0.05$ was considered significant.

RESULTS

Nutritional rehabilitation: Table 1 shows the anthropometrics parameters of the children HIV infected and HIV negative at the beginning of our study.

The nutritional changes pre/post improved in all children, more significantly in the group who received Spirulina. These changes among treatment groups are reported in Table 2. This improvement corresponds to an increment of weight which was on the average 15 g a day in HIV infected children and on average 25 g a day for HIV negative children both treated with Spirulina plus traditional meals. The differences within groups were statistically significant considering the differences in the nutritional status changes across the groups, but this difference was less significant in the control group (10 and 20 g/day, respectively).

The HIV negative children who are nourished with Spiruline plus traditional meals had a more significant ($p<0.0003$) decrement of WHZ parameter than the HIV-infected children ($p=0.0004$). At the end of the eight weeks of the treatment, nutritional status normalized for the majority of children, WHZ parameter decreasing from -2.26 to -0.93; the average of nutritional status of the HIV-infected children was not so good as the average for

Table 1: Anthropometric parameters of the children subjected to the study

	Groups				Variance
	A	B	C	D	analysis
Age (months)	14.37±6.4	15.54±5.3	15.19±4.35	14.96±5.9	p=0.789
Height (cm)	69.64±1.1	69.72±5.8	68.24±4.5	69.84±5.8	p=0.370
BP	10.65±1.1	10.14±0.9	10.37±1.0	10.40±1.0	p=0.124
Weight (kg)	6.05±1.1	5.91±1.2	6.10±1.2	5.98±1.1	p=0.882
HAZ	-2.59±1.5	-2.88±1.3	-3.23±1.5	-2.64±2.1	p=0.758
WHZ	-2.94±-0.7	-2.87±1.0	-2.42±1.02	-2.88±0.9	p=0.038
WAZ	-3.83±0.9	-4.10±0.8	-3.99±0.9	-3.88±1.0	p=0.503

*HAZ=Height for age z-score; WHZ=Weight for height z-score; WAZ=Weight for age z-score, BP=Brachial Perimeter

Table 2: Nutritional status at the beginning (1) and at the end of the study (2)[a]

	Groups			
	A	B	C	D
WHZ1	-2.94±0.73	-2.87+/-1.00	-2.42±1.02	-2.88±0.95
1→2	p=0.000*	p=0.004*	p=0.065*	p=0.000*
WHZ2	-1.70±1.62	-2.23+/-1.01	-2.00±0.99	-2.58±1.53
WHZ2/ (WHZ1+WHZ2)	42.1%	22.2%	17.3%	10.41%
WAZ1	-3.83±0.91	-4.10+/-0.83	-3.99±0.9	-3.88±0.90
1→2	p=0.000*	p=0.007*	p=0.013*	p=0.000*
WAZ2	-2.98±1.16	-3.59+/-0.91	-3.45±1.0	-3.59±1.14
WAZ2/ (WAZ1+WAZ2)	22.19%	14.63%	13.53%	7.47%

[a]WHZ1=Weight for height z-score at beginning of the study; WHZ2=Weight for height z-score at the end of the study; WAZ1=Weight for age z-score at the beginning of study; WAZ2=Weight for age z-score at the end of the study.
* 1→2 Student t test

Table 3: Biological parameters of the children made at the beginning (1) and at the end of the study with Spiruline (2)

	Children HIV negative treated with Spiruline No. 46		Children HIV-infected treated with Spiruline No. 44	
	Mean	SD	Mean	SD
Red Cell mm^3 (1)	3.64	0.82	3.34	0.63
1→2	p=0.000*		p=0.000*	
Red Cell mm^3 (2)	3.96	0.65	3.72	0.54
Hb g dL^{-1} (1)	8.53	1.54	8.01	1.10
1→ 2	p=0.000*		p=0.000*	
Hb g dL^{-1} (2)	9.73	0.83	9.42	1.20
Leukocytes x1000 (1)	11.75	3.02	13.00	3.00
1→ 2	p=NS*		p=0.000*	
Leukocytes x1000 (2)	12.00	5.51	10.00	4.03
Lymphocytes% (1)	38.41	3.59	39.09	4.48
1→ 2	p=NS*		p=0.000*	
Lymphocytes% (2)	40.87	1.64	41.64	1.29
Neutrophil% (1)	51.04	4.40	49.82	5.30
1→ 2	p=NS*		p=0.000*	
Neutrophil% (2)	47.85	2.50	42.83	0.83

* Student t-test

HIV negative children. The index weight for age WAZ at the end of our study confirmed that the severe malnutrition was corrected by this protocol of treatment, more significantly in the Spiruline group. The percentage of WHZ and WAZ are reported in Table 2. The association of Spiruline plus traditional meals gave in HIV infected children gain of 22.2 and 14.63%, respectively, against 10.41 and 7.47%, respectively using alone traditional meals. In HIV negative children the gain was 42.1 and 22.19%, respectively when Spiruline was added to traditional meals and 17.3 and 13.53% when they were alimented traditionally alone.

The analysis of this data shows that the best improvement was observed in the group of HIV-infected children (Table 3). This response to the Spiruline supplement was more significant than the increment of weight and height showed by WHZ and WAZ parameters. The median number of the red cells and the median concentration of haemoglobin, allowed to diagnose an anaemia ($3.59\,10^6$ mm^{-3} of red cells and 8.44 g dL^{-1} of haemoglobin) for all the children. Children who were HIV-infected were more anaemic ($3.34\,10^6$ mm^{-3}) than the children HIV negative ($3.64\,10^6$ mm^{-3}). At the end of the eight weeks, the anaemia was slightly corrected for all HIV-infected and HIV negative children who remained nevertheless anaemic ($3.92\,10^6$ mm^{-3}).

The compliance to treatment was excellent and any children drop out. They come to weekly appointments, but only the first and the last visit (eight weeks) were considered in the final evaluation.

Table 4: The Nutrition Composition for 100 g of cultivated Spiruline to the Medical Center St Camille in comparison of the given values in the literature[12]

	Our results	Green Flamant values
Water content (%)	4.87	3-7
Ash (%)	10.38	7-13
Vegetal fiber (%)	7.81	8-10
Lipid (%)	6.00	6-8
Protein (%)	57.10	55-70
Glucide (%)	13.84	15-25

Table 5: Physicochemical composition of the Spiruline with the time

Analysed sample	T_0 (1st day)	T_1 (1st month)	T_2 (2nd month)	T_3 (3rd month)	T_4 (10th month)
Protein (%)	57.10	56.22	54.69	52.28	49.22
Formic index (mL NaOH)	4.35	4.20	4.47	5.19	4.81
Total sugars (%)	12.77	16.43	19.59	18.16	16.07
Reductive sugars (%)	1.07	2.52	2.17	1.56	1.62
Fat matter (%)	6.00	7.19	6.69	5.92	7.25
Fatty acids (mg NaOH/g)	6.60	6.00	7.50	6.90	10.20
pH	6.53	6.56	6.36	6.78	7.33
Humidity (%)	4.87	4.86	5.01	4.83	4.42
Ash (%)	10.76	12.12	10.19	11.46	14.44
Phycocyanin (%)	9.76	7.46	6.12	7.32	4.46
Energetic value (kcal/100g)	338.0	360.0	363.0	340.00	331.00

Student t test for paired data : $T_0 \rightarrow T_1$: p = 0.273; $T_0 \rightarrow T_2$: p = 0.310; $T_0 \rightarrow T_3$: p = 0.763; $T_0 \rightarrow T_4$: p = 0.625

Table 6: Fatty acid composition of Spirulina strain from Burkina Faso

Fatty acid	Wt.% of total fatty acid
Palmitic acid, 16:0	28.04
Palmitoleic acid, 16:1 (9)	2.69
Stearic acid, 18:0	13.44
Oleic acid, 18:1 (9)	18.88
Linoleic acid, 18:2 (9, 12)	21.87
∝Linolenic acid, 18:3 (6, 9, 12)	15.08

Chemical analysis: The values of the composition of the Spiruline of the CMSC of Ouagadougou is in the interval of values of the international firm Green Flamant[12]. The composition of our Spiruline proves the good quality of the Spiruline of the CMSC (Table 4). The only difference is situated at the level of the value of the glucides. The lesser content in glucides of the analysed Spiruline in our culture conditions was near the one of Sautier and Tremolieres[13], that in 1975 found a value of 12.4% on the cultivated Spiruline in laboratory. The quality of the Spiruline with the time-in the first three months of storage

did not show significant changes. For longer period of storage some significant changes were detected, such as a decrease of protein content and an increase of pH value.

The lipid composition of the Spiruline growth in Burkina Faso is listed in Table 5. The fatty acid content is represented by palmitic, linoleic, oleic, ∝-linolenic, stearic and palmitoleic acids.

DISCUSSION

After eight weeks of study, HIV infected and HIV negative children treated with Spiruline plus traditional meals appear improved, their weight grew and many of them appear less anaemic. This improvement was less significant in the control group, who received only traditional meals. The enrolment of this group could appear unethical among these severely malnourished children, but it was organized choosing randomly a control group between children whose mothers did not accept the protocol study, so they were treated only with traditional meals.

The results of this study prompted us to continue the culture of Spirulina in the CMSC of Ouagadougou in order to utilize the biochemical composition and the beneficial action of this cyanobacterium, which may be considered as an alimentary integrator for undernourished children. In the context of weak intake of proteins, 10 g a day by inhabitant in Africa against 29 g in Latin America and 63 g in the industrialized countries, the integration of traditional meal with Spiruline improve the nutritional and micronutrient requirement for undernourished children[14].

Moreover, this result shows that the Spiruline is also effective for the haematopoiesis especially for the persons infected by HIV in accordance with the data of the international literature[8,15]. This may be due to the iron content of Spiruline supplement[16], which correct the anemia due to deficient iron intake.

Since at the beginning of this study the number of leucocytes: 12.370 mm^{-3}) with relative granulocytopenia was found elevated in the HIV-infected children (49.8%) (Table 3), the increase of lymphocyte number in HIV-infected children who received eight weeks of Spiruline, confirm the immune modulation of this cyanobacterium. This mechanism may be due to the high amount in the lipid fraction of ω-6 derivative, namely ∝-linolenic acid[17]. This exclusive presence of ω-6 represents a metabolic gain, since desaturase enzyme could be deficient in the undernuorished children[18].

The growth recovery is slower than the weight recovery and this could be determined by the diarrhoea, which was present at the beginning of treatment of these children[19]. In fact, in our study regarding a period of eight weeks, the variations of weight were more significant owing to the liquid content dehydratation associated with malnutrition. WHZ was smaller in HIV-infected children in comparison with HIV negative children (Table 1), since they frequently show diarrhoea associated with HIV infection.

One could object that this study assigned the Spiruline treatment mainly to children with HIV-infected and non infected, who showed more severe alterations of WHZ and WAZ values. This choice could alter the analysis of results, however the unexpected increment in weight with the use of Spiruline confirms the opportunity for continuing the use of this supplement in undernourished children. Previous study made by Branger *et al.*[20] in Burkina Faso did not show a significant improvement adding Spiruline to traditional meal but, as considered by the same authors, the scarce results which they obtained could be due to the quantity of Spiruline, which was half that used in our study (5 vs. 10 g). Moreover, the present study is more conclusive than the one realized in Dakar by Alling *et al.*[21], in which the gain of weight was inferior, probably due also in this case, to a reduced supplement in Spiruline.

The anthropometrics characteristics of studied children varied little according to the sex (Table 2), but they were different according to the nutritional and serologic status. This observation is the same as the one of Kelly *et al.*[22] in undernourished HIV-infected children with persistent diarrhoea. In St Camille Medical Center a prevalence of 0.82% for the kwashiorkor, 95.96% for the marasma and 3.22% for the kwashiorkor plus marasma was found, which correspond to the effect of HIV infection on nutritional status of children in Burkina Faso[23]. Moreover, the screening of these aforesaid children at the Medical Center confirmed a 24.44% of HIV-infected children. This frequency is less than the prevalence of 27% found at Bobo-Dioulasso (Burkina Faso) by Prazuck *et al.*[24] and of 28.6% in Togo by Atakouma *et al.*[25]. The strong prevalence the kwashiorkor and/or marasma is characteristic of sub-Saharan Africa, where maize and millet are the staple. An high intake of linoleic acid in a diet deficient in other polyunsaturated fatty acids and in riboflavin results in these countries in high tissue production of prostaglandin E2, which in turn causes inhibition of the proliferation and cytokine production of Th1 cells, mediators of cellular immunity[26]. Diet-associated inhibition of the Th1 subset is a major contributor to the high prevalence of these clinical pictures of malnutrition in sub-Saharan areas.

The infection with HIV among the undernourished children worsens the situation and highlights the problem

of the charge of medical and nutritional structures. This study could suggest a preliminary solution with Spiruline for accelerating the nutritional rehabilitation before starting an antiretroviral treatment.

This study shows that the malnutrition remains a public health problem in Burkina Faso. The consequence of the malnutrition in association with HIV infection represent a global problem, which affects the morbidity as well as the mortality. Awaiting for the enrolment in treatment protocols of these undernourished children with different pathologies associated to HIV, the persons in charge at public health service and epidemiologists should work very synergically with nutritionists, bacteriologists and virologists in order to fight efficiently against malnutrition and particularly the paediatric malnutrition associated to the HIV.

The Spiruline has 57.10% of protein and an high amount of ω-6 lipid component which support an efficient recovery of the precarious immune system of these children. These characteristics confirm the benefit of the supplement Spiruline (this association gave a gain of 25 g a day in HIV negative children). According to the instructions which the mothers received, an involvement of the families of the undernourished children and of the whole community is essential to control the great prevalence of the malnutrition and HIV infection in African countries.

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